The Effect of garlic extract (*Allium sativum*) on the hatching rate of sea bass eggs (*Lates calcarifer*)

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Abstract. The purpose of this study was to determine the effectiveness of giving garlic extract *(Allium sativum)* to the hatching rate of sea bass eggs *(Lates calcarifer).* This research was conducted in February-March 2024 at the Brackish Water Aquaculture Center Ujung Batee, Aceh Besar. The method used in this study was a completely randomized design (CRD) consisting of 5 treatments and 4 replicates. The concentration of garlic used includes treatment A which is 0 (control), treatment B 1.000 ppm, treatment C 1.500 ppm, treatment D 2.000 ppm, and treatment E 2.500 ppm. The results of this study showed the hatching rate (HR) of eggs ranged from 30.75-92.75% and the survival rate (SR) ranged from 20.75-89.25%. Based on the ANOVA test, the addition of garlic extract has a real significant effect ($P<0.05$) on the hatching rate of seabass eggs and the survival of sea bass larvae. Soaking eggs in garlic extract was found to be effective in inhibiting fungal attacks on sea bass eggs that had previously been infected with fungi. This is proven by the high percentage of egg hatching and survival rate of sea bass larvae. In this study, treatment D (2000 ppm) was the best treatment which produced a hatching rate percentage of 92.75%, with a survival rate 89.25%.

1 Introduction

The sea bass (*Lates calcarifer*), also known as Asian sea bass or barramundi, is a fish species with high economic value, both for domestic and international consumption. Sea bass aquaculture has become a commercially viable venture [1]. The success of sea bass aquaculture greatly depends on the availability of high-quality and abundant broodstock, eggs, and larvae. One of the key factors supporting this is a high hatching rate of eggs and larval survival, ensuring a continuous supply of sea bass fry [2].

The production of sea bass fry has been steadily increasing year after year. In 2021, the total production volume of sea bass fry was 9,207,000, which saw a significant increase to 143,791,000 in 2023 [3]. It makes the demand for sea bass fry is currently not sufficient, therefore it is necessary to make improvements in fry cultivation that can increase production

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for sea bass fry. However, one of the factors that causes mass mortality in eggs and results in a lack of fry is greatly influenced by environmental factors [4]. The availability of sea bass fry often faces problems in hatchery, namely the attack of the *Saprolegnia* sp. fungus found in fish eggs, which can reduce the hatching rate of eggs. This fungal infection can be triggered by several factors, including high egg density and poor water quality [5, 6].

The chemicals commonly used to prevent and treat fungal attacks on fish eggs include Methylene blue, formalin, NaCl, Malachite green, and Potassium Permanganate. Continuous use of these chemicals should be avoided as they can have harmful effects on organisms and the environment, and they are also relatively expensive and difficult to obtain [7]. Therefore, this study attempts to use natural materials that have less harmful impacts. One alternative to treat fungal attacks on fish eggs is to use natural ingredients such as garlic, which is known to have antifungal properties [6].

Garlic contains essential oils that have antibacterial and antiseptic properties. In addition, garlic also contains allicin compounds that can appear when garlic is cut. Allicin is also useful as an antibacterial, antifungal, antioxidant and anticancer [9, 10]. The use of garlic extract has been carried out in several studies, research by [11] stated that garlic extract can minimize the attack of *Saprolegnia* sp. fungus on tengadak fish eggs. Research by [12] also showed that the best concentration of garlic extract was 2,000 ppm with an average hatching rate of milkfish eggs of 93%. However, the use of garlic extract on sea bass eggs and the optimal dose to minimize fungal infections in sea bass eggs is not yet known. Therefore, this study is important to be carried out with the aim of determining the effectiveness of giving garlic extract (*Allium sativum*) on the hatching rate of sea bass eggs (*Lates calcarifer*).

2 Materials and methods

2.1 Time and places

This research was conducted for 30 days, in February-March 2024 at the Ujung Batee Brackish Water Aquaculture Center, Durung Village, Aceh Besar.

2.2 Materials and equipment

The materials and equipment used for this research were sea bass eggs, garlic extract, blender, Wattman filter paper, rotary evaporator, glassware, container for cultivation, aerator, ethanol, and dropper pipette.

2.3 Research design

This research used a Completely Randomized Design (CRD) method with 5 treatments and each treatment was replicated 4 times. Sea bass fry was reared based on the guidelines of the Institutional Animal Care and Use Committee (IACUC, 2018) and have ethical approval B/KEH/034SN/XII/2022 from the animal ethic committee of Marine and Fiseheries Faculty, Universitas Syiah Kuala. Determining the dose of garlic extract used in this study refers to the treatment used by [12]:

- Treatment A: Addition of garlic extract with a concentration of 0 ppm (control)
- Treatment B: Addition of garlic extract with a concentration of 1,000 ppm
- Treatment C: Addition of garlic extract with a concentration of 1,500 ppm
- Treatment D: Addition of garlic extract with a concentration of 2,000 ppm
- Treatment E: Addition of garlic extract with a concentration of 2,500 ppm

2.4 Research procedure

2.4.1 Preparation of garlic (Allium sativum) extract

The preparation of garlic extract refers to the research of [12]. The process of preparing garlic extract (*Allium sativum*) is obtained through a series of processes. First, the garlic cloves are peeled. Then, they are washed thoroughly and sliced thinly to reduce water content and facilitate grinding. The sliced garlic is then air-dried for 7 days at room temperature. After 7 days, the dried garlic is weighed and ground into a powder using a blender. The amount of garlic powder used in this study was 250 grams.

The powder of garlic (*Allium sativum*) was extracted using the maceration method with 96% ethanol as the solvent. The mixture was allowed to macerate for at least 2 x 24 hours, then filtered using filter paper to separate the solid material from the solution. The garlic extract was evaporated using a rotary evaporator at 65°C for approximately 30 minutes until a concentrated solution was obtained. The concentrated garlic extract was stored in a freezer until use. Garlic extracts used for soaking sea bass eggs were prepared at concentrations of 1,000 ppm, 1,500 ppm, 2,000 ppm, and 2,500 ppm.

2.4.2 Preparation of test vessels

The containers used in this study were 5-liter plastic jars, each filled with 2 liters of water. A total of 15 units were used as infection containers, egg soaking containers, and larval rearing containers. Before use, the containers were washed with clean water and dried to prevent contamination. They were also equipped with aeration to supply oxygen to each hatching media [6].

2.4.3 The fungal infection

The seabass eggs used in this study were obtained from semi-natural spawning conducted at the Brackish Water Aquaculture Center (BPBAP) Ujung Batee, Aceh Besar. A total of 100 fertilized healthy eggs and 25 eggs infected with fungi were used. Healthy eggs were left to soak for 1 hour until they were infected with fungi [13]. Both the fertilized healthy eggs and the fungus-infected eggs were transferred to soaking containers that had been previously filled with garlic extract at the specified dosage. The soaking process was carried out for 5 minutes for each treatment [12]. Subsequently, the eggs were removed and transferred to hatching containers. The condition of the eggs was observed from the moment they were soaked in the garlic extract solution until they hatched.

2.4.4 Parameter of research

The parameters observed in this research: Hatching Rate (HR) of sea bass eggs and Survival Rate (SR) of sea bass fry.

3 Result and discussion

The results of the research that has been conducted on testing the hatching rate of eggs and the survival rate of sea bass larvae (*Lates calcalifer*) soaked in garlic extract solution (*Allium sativum*) are presented in Table 1.

Table 1. The effect all of treatment on Hatching Rate (HR) of eggs and Survival Rate (SR) of sea bass larvae.

Treatments	Parameter	
	The hatching rate of *Lates calcarifer*'s eggs (%)	**The survival rate of *Lates calcarifer*'s larvae (%)**
A (control)	30.75 ± 2.98^{a}	20.75 ± 1.70^{a}
B (1.000ppm)	79.5 ± 1.29^{b}	74.25 ± 3.30^{c}
C (1.500ppm)	83.25 ± 1.25^{c}	77.75 ± 1.25^{c}
D (2.000ppm)	92.75 ± 0.95^{d}	89.25 ± 0.95^{d}
E (2.500ppm)	75.75 ± 1.70^{b}	66.00 ± 4.08^{b}

The values with different superscript letters in a column are significantly different ($p<0.05$)

The ANOVA test revealed a significant effect ($P<0.05$) of garlic extract (*Allium sativum*) treatment on the hatching rate of seabass (*Lates calcarifer*) eggs. BNJ test showed that treatment D (2,000 ppm) showed the highest hatching rate at 92.75%, which was significantly different ($P<0.05$) from all other treatments. This is presumably because treatment D, with the application of garlic extract, effectively inhibited fungal attacks on the fish eggs. [12] stated that the use of antimicrobials on eggs can prevent fungal growth, but at higher concentrations, it can damage the chorion tissue of the eggs, causing them to shrink and ultimately fail to hatch.

Garlic's defense mechanism: it activates a defense mechanism that produces a sulfur-containing compound called allicin. Allicin is a potent antimicrobial agent. However, it is quite unstable and quickly breaks down into other sulfur compounds like DAS, DADS, DATS, DATTS, vinyldithiin, ajoene, and others. All of these compounds exhibit various health benefits, including antidiabetic, antibacterial, antifungal, antimicrobial, and anticancer properties [18]. Allicin can penetrate not only cell membranes but also organelle membranes like mitochondria, leading to organelle damage and cell death. Additionally, it can alter the expression of specific genes involved in cellular responses to drugs, redox processes, pathogenesis, and cellular starvation [19]. Garlic's antifungal activity is attributed to its ability to inhibit spore germination and hyphal growth both in living organisms and in laboratory conditions. Its antimicrobial effects also stem from its rapid reaction with thiol-containing enzymes in microorganisms, a characteristic common to thiosulfinate compounds [18, 20].

According to [14], the results of phytochemical screening also show that garlic contains active compounds such as flavonoids, alkaloids, phenolics, and tannins. [15] also added, that flavonoids and alkaloids function as antifungal agents, thus inhibiting fungal growth and treating fungal-infected eggs. The results of the hatching rate align with the research conducted by [12] on the effect of garlic extract (*Allium sativum*) on the hatching rate of milkfish (*Chanos chanos*) eggs. The results of that study showed a hatching rate of 93% with 2,000ppm extract treatment.

Treatment A (control), or treatment without garlic extract (*Allium sativum*), showed the lowest hatching rate of 30% and was significantly different ($P<0.05$) from all other treatments. This is presumably because in this treatment, the eggs were not given garlic extract, allowing fungi to quickly infect and rot the eggs, ultimately preventing them from hatching. This is in line with the research of [11] on the effectiveness of garlic extract against *Saprolegnia* sp. fungus in snakehead fish eggs, where the control treatment (without garlic extract) resulted in a hatching rate of 44.67%. This is further supported by the statement of

[16] that eggs not soaked in antifungal agents rely solely on the chorion to resist fungi, resulting in a high number of unhatched eggs.

Based on the ANOVA test conducted, the garlic extract (*Allium sativum*) had a significant effect ($P<0.05$) on the survival rate of seabass (*Lates calcarifer*) larvae. Post-hoc BNJ test showed that treatment D (2,000 ppm) yielded the highest survival rate of 89.25%, which was significantly different ($P<0.05$) from all other treatments. This is presumably because the given dose was optimal in inhibiting fungal growth, and the antifungal properties of garlic extract were able to protect the eggs from fungal infection, allowing them to develop into larvae. On the other hand, treatment A (control) had the lowest survival rate of 20.75% and was significantly different ($P<0.05$) from all other treatments. The low survival rate in treatment A (control) was due to the lack of garlic extract, which left the eggs unprotected, leading to fungal infections and subsequent mortality. This is supported by [18], who stated that garlic extract contains the compound allicin, which belongs to organosulfur compounds, and can enhance the immune system, thus improving larval survival. When milkfish eggs were soaked in garlic extract, the allicin content in the garlic penetrated the eggs, enhancing their resistance and positively influencing larval growth.

4 Conclusions

Soaking sea bass (*Lates calcarifer*) eggs in garlic extract (*Allium sativum*) has been effectively applied to inhibit fungal attacks in sea bass hatchery. Based on the ANOVA test, the application of garlic extract had a significant effect ($P<0.05$) on the hatching rate of sea bass eggs. The highest hatching rate percentage was found in treatment D (2,000 ppm) at 92.75%. The highest larval survival rate was also found in treatment D, with a value of 89.25%.

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